

**Intestinal Colonization with Extended-Spectrum Cephalosporin- and  
Colistin-Resistant *Enterobacteriaceae* in HIV-Positive Individuals in Switzerland:  
Molecular Features and Risk Factors**

João Pires,<sup>1,2</sup> Odette J. Bernasconi,<sup>1,2,§</sup> Christoph Hauser,<sup>3,§</sup> Regula Tinguely,<sup>1</sup>

Andrew Atkinson,<sup>3</sup> Vincent Perreten,<sup>4</sup> Valentina Donà,<sup>1</sup> Andri Rauch,<sup>3</sup>

Hansjakob Furrer,<sup>3</sup> and Andrea Endimiani<sup>1\*</sup>

<sup>1</sup>Institute for Infectious Diseases (IFIK), University of Bern; <sup>2</sup>Graduate School of Cellular and  
Biomedical Sciences, University of Bern, Bern; <sup>3</sup>Department of Infectious Diseases, Bern  
University Hospital, University of Bern, Bern; <sup>4</sup>Institute of Veterinary Bacteriology, Vetsuisse  
Faculty, University of Bern, Bern, Switzerland

§ Contributed equally to the work

**Short running title:** HIV-positive colonized with MDR *E. coli*

**\*Corresponding author:**

Prof. Andrea Endimiani MD, PhD

Institute for Infectious Diseases, University of Bern

Friedbühlstrasse 51, CH-3001, Bern, Switzerland

Phone: +41-31-632 8 632; Fax: +41-31-632 8 766

Emails: [andrea.endimiani@ifik.unibe.ch](mailto:andrea.endimiani@ifik.unibe.ch); [aendimiani@gmail.com](mailto:aendimiani@gmail.com)

26 Sir,  
27 The increasing rates of gut colonization by extended-spectrum cephalosporin-resistant (ESC-R-)  
28 and/or colistin-resistant *Enterobacteriaceae* (COL-R-Ent) carrying the plasmid-mediated *mcr-1*  
29 gene in healthy humans raises serious concerns [1, 2]. Nevertheless, colonization prevalence and  
30 risk factors associated with HIV+ individuals are unknown. This population undergoes a decrease  
31 in CD4+ T cells in the gut-associated lymphoid tissue which is linked with a microbiota dysbiosis  
32 possibly favoring colonization [3]. The aim of this pilot study was to better understand this  
33 phenomenon to improve the management of these patients.

34 Between March 2015 and April 2016, 101 HIV+ individuals on suppressive anti-retroviral  
35 therapy (ART) donated a stool sample and filled out a questionnaire (Table S1 and S2). To detect  
36 ESC-R-, carbapenem-, and/or COL-R-Ent, stools were enriched in broth and plated on selective  
37 agar plates [1, 2]. Species identification was achieved by using the MALDI-TOF MS (Bruker  
38 Daltonics, Leipzig, Germany). MICs were obtained using the Sentitre™ GNX2F plate (Trek  
39 Diagnostic Systems, Independence, OH, USA).  $\beta$ -lactamase genes were identified using both  
40 CT103XL microarray (Check-Points, Wageningen, Netherlands) and PCR/sequencing [1, 2]. COL-  
41 R-Ent were screened for *mcr-1/2* and PmrAB two-component system with PCR/sequencing [1].  
42 Whole genome sequencing was carried out with MinION (Oxford Nanopore, Oxford, UK) [1].  
43 Clonality was assessed with standard MLST (<http://mlst.ucc.ie/mlst/dbs/Ecoli>) and phylogenetic  
44 grouping [2]. The PBRT kit (Diatheva, Cartoceto, Italy) was used to type plasmids. Conjugation  
45 was performed using *E. coli* JF33 [1]. Univariate analysis was performed to compare colonized and  
46 non-colonized subjects (GraphPad Prism software, version 7.0; La Jolla, CA, USA). Continuous  
47 variables were analyzed using Mann–Whitney U test, whereas categorical variables with Fisher's  
48 exact test.

49 Seven volunteers (6.9%) resulted colonized with ESC-R *E. coli* (other *Enterobacteriaceae* were  
50 not detected). This data is consistent with that reported in the healthy population in Switzerland and  
51 in other European countries [1, 2]. However, when looking into *Enterobacteriaceae* causing

infections in HIV+ subjects, the prevalence of ESC-R-Ent dramatically increased to 50%, though these studies were either conducted in countries with high occurrence of these multidrug-resistant organisms (MDROs) or have enrolled individuals not under ART [4 , 5].

Most ESC-R *E. coli* recovered in the present study were CTX-M-15 producers and associated with F plasmids (Table 1) [2]. Each isolate yielded an individual sequence type (ST), including the high risk clones (HiRC) ST131, ST73, ST405 and ST410 that might be associated with the frequent contact of HIV+ people with the healthcare facilities [2].

Four volunteers (4.0%) were also colonized with COL-R *E. coli* (other *Enterobacteriaceae* were not detected), of which one (31349) was of B1-ST5 and *mcr-I*-positive. The remaining three strains failed to demonstrate a plasmid-mediated mechanism of colistin resistance after conjugation experiments [1]. Analysis of the PmrAB system indicated that these strains had amino acid substitutions possibly driving colistin resistance (Table 1) [1]. Demographic, clinical and epidemiological data about the four volunteers colonized with COL-R *E. coli* are shown in Table S1. Notably, volunteer 31349 was a 58 year-old male who in the last year was hospitalized in Switzerland and received antibiotics, but he did not traveled anywhere.

Screening of raw genome data by PlasmidFinder-1.3 and ResFinder-2.1 indicated that *E. coli* strain 31349 possessed IncF, IncL, and IncX4 plasmids together with the *mcr-I* (no other resistance genes were detected) [1]. *Mcr-I* was located in a 33.3 Kb plasmid that shared >99% identity ( $\leq 30$  mismatches) with IncX4 plasmids reported in *E. coli* from pig faeces in China (GenBank: KX254343) and from river water in Switzerland (GenBank: KZ129783). This is the first report of *mcr-I*-carrying *Enterobacteriaceae* in the gut of HIV+ people and highlights the global dissemination of this life-threatening resistance mechanism [1]. Despite the fact this COL-R strain carrying *mcr-I* was pan-susceptible to all antibiotics, the detection of this gene in a population carrying ESBL-producing HiRC is of great concern because these MDROs can further acquire the *mcr-I* and disseminate across different settings.

77        Regarding the univariate analysis, only the total CD4 cells count at the time of stool sampling  
78        was significantly lower among the colonized than those non-colonized subjects (median 301 vs. 706  
79        cells/ $\mu$ l;  $P=0.02$ ; Table S2 and Figure S1). This observation may have important implications for  
80        empirical therapy when HIV+ individuals have serious bacterial infections. However, given the  
81        small sample size, our study lacks statistical power. This hinders meaningful multivariable analysis  
82        and potentially other associations in the univariate analysis. Additionally, it is possible that our  
83        results are representative only for a country (Switzerland) with low-prevalence of ESC-R *E. coli*  
84        colonization in the general population alongside HIV+ people under successful ART. Finally, as  
85        already reported in the general population, travelling to South-East Asia ( $P=0.07$ ) and  
86        hospitalization abroad ( $P=0.13$ ) could also be potentially correlated with ESC-R-Ent colonization  
87        [1, 2]. It is important to note that we were unable to include a control group due to the lack of  
88        information about HIV status and CD4 cell count of healthy people.

89        This is the first study analyzing the presence of MDR *Enterobacteriaceae* in the intestinal tract  
90        of HIV+ people. When under ART, these subjects seem to acquire such pathogens as likely as the  
91        general population. However, the identification of HiRC underlines the potential for developing  
92        future difficult-to-treat extra-intestinal infections. Moreover, the identification of a strain carrying  
93        *mcr-1* on a plasmid with high similarity with others present in food animals and environment  
94        highlights potential sources of acquisition of these pathogens. Finally, a low total CD4 count might  
95        be an additional risk factor favoring intestinal colonization. Elucidating this phenomenon with a  
96        larger cohort will be crucial for a better management of HIV+ patients.

97    **ACKNOWLEDGMENTS**

98    We thank Sara Kasraian for the technical help and both Melanie Lacalamita and Daniela Hirter for  
99    collecting epidemiological and clinical data. Data collection was facilitated by the Swiss HIV  
100   Cohort Study that is supported by the Swiss National Science Foundation (SNF; grant number  
101   148522). João Pires is a PhD student (2014-2017) supported by the SNF. Odette J. Bernasconi is a  
102   PhD student (2015-2018) supported by the Hans Sigrist Foundation (Bern, Switzerland).

103

104   **FUNDING**

105   This work was supported by the Swiss National Science Foundation (SNF; grant number 153377 to  
106   AE).

107

108   **COMPETING INTERESTS**

109   None declared

110

111   **ETHICAL APPROVAL**

112   The study was approved by Kantonale Ethikkommission Bern (KEK): *Schweizerische HIV*  
113   *Kohortenstudie* (No. 21/88).

114   **REFERENCES**

- 115   [1] Bernasconi OJ, Kuenzli E, Pires J, Tinguely R, Carattoli A, Hatz C, et al. Travelers Can Import  
116   Colistin-Resistant Enterobacteriaceae, Including Those Possessing the Plasmid-Mediated *mcr-1*  
117   Gene. Antimicrob Agents Chemother. 2016;60:5080-4.
- 118   [2] Pires J, Kuenzli E, Kasraian S, Tinguely R, Furrer H, Hilty M, et al. Polyclonal Intestinal  
119   Colonization with Extended-Spectrum Cephalosporin-Resistant *Enterobacteriaceae* upon Traveling  
120   to India. Frontiers in Microbiology. 2016;7.
- 121   [3] Zilberman-Schapira G, Zmora N, Itav S, Bashiares S, Elinav H, Elinav E. The gut microbiome  
122   in human immunodeficiency virus infection. BMC Medicine. 2016;14:1-11.
- 123   [4] Padmavathy K, Padma K, Rajasekaran S. Extended-spectrum  $\beta$ -lactamase/AmpC-producing  
124   uropathogenic *Escherichia coli* from HIV patients: do they have a low virulence score? Journal of  
125   Medical Microbiology. 2013;62:345-51.
- 126   [5] Marwa KJ, Mushi MF, Konje E, Alele PE, Kidola J, Mirambo MM. Resistance to  
127   Cotrimoxazole and Other Antimicrobials among Isolates from HIV/AIDS and Non-HIV/AIDS  
128   Patients at Bugando Medical Centre, Mwanza, Tanzania. AIDS Research and Treatment.  
129   2015;2015:8.

130

131